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alpha gamma

The goal of this project is to investigate specificity and high affinity of small peptide ligands to their receptor targets. A specific goal is to determine the conformation of small natural peptide ligands. In the last year two peptides were investigated by 2D-NMR, α-conotoxin SI, which targets to the nicotinic acetylcholine receptor (but discriminates between the Torpedo and mammalian receptors), and the "sleeper" peptide, which contains 5 residues of γ-carboxyglutamate. In addition, significant progress was made towards understanding how small cysteine-rich peptide ligands might be specifically folded in *Conus* venom ducts.

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SECURITY CLASSIFICATION OF THIS PAGE

Progress Report on Contract N00014-88-K-0178
Principal Investigator: Baldomero M. Olivera
Contractor: University of Utah, Salt Lake City, UT 84112
Contract Title: Targeted Peptide Specificity
Start Date: 1 February 1988

Research Objective: To investigate small peptide ligands with high affinity and specificity for their receptor target. The two general goals are to use natural peptide ligands from Conus venom as a model system to understand the molecular basis of the specificity and high affinity in the ligand receptor interactions achieved in this system. A second long range goal is to use molecular genetics to develop synthetic ligands for predetermined targets.
Keywords: Cone Snails, Conus textile. (AW)

A major goal of this work is to understand the interactions between small peptide ligands and their receptor target. This investigation is based on results that have already been obtained in a natural system, the small peptide toxins present in the venoms of cone snails. The venomous cone snails are a large family of predatory gastropods, highly specialized to feed either on fish (ca. 50 species), other snails (ca. 50 species) or a variety of marine worms (ca. greater than 200 species). The biological strategy that has allowed these snails to be among the major predators of coral reef communities is the production of very small (10-30 amino acids) peptides, usually highly disulfide-bonded which target to a variety of physiologically relevant receptor targets. Although these venoms are very complex, we have focused on three groups of peptide toxins, the α, μ and ω-conotoxins which target to nicotinic acetylcholine receptors, sodium channels and voltage sensitive Ca channels respectively. All of these peptides are 20-30% cysteine Cys (always present as disulfide bonds) and are 13-27 amino acids in length.

Recently, using a toxin from the snail-hunting cone, Conus textile, we have gained insight into the molecular genetic strategy of the snails for generating the bewildering array of small biologically active cystine-rich peptides found in Conus venoms. In collaboration with the laboratory of David Hillyard, the genes for one of these small disulfide rich peptides, the "King-Kong" peptide was cloned and sequenced. The King-Kong peptide is 27 amino acids long; the primary translation product has proven to be 78 amino acids long. The coding sequence for the final peptide is found at the C-terminus, and is immediately followed by a stop signal. The first ca. 20 amino acids of the N terminus appear to be a reasonable signal sequence. However, the leader sequence which is presumably excised as the final peptide is generated is far longer than for toxins characterized from other phylogenetic groups (i.e., snake neurotoxins or scorpion toxins). The most surprising results in this investigation however was the discovery that the transcript for this peptide was only one member of a family of related transcripts, in which the N terminal excised region was highly conserved (> 90% AA sequence identity). However, the sequences of the final peptide generated from each transcript were highly divergent from the original King-Kong peptide sequence. Three members of the family were sequenced, and in each case, there was very little amino acid homology at the C terminal region except for Cys residues, which were perfectly conserved. Thus, in this family of transcripts there are constant and hypervariable regions. The constant regions include the 51 amino acid excised N terminal region and the cysteine residues in the final peptide; the hypervariable regions comprise the inter-cysteine sequences of the final peptide. In the region coding for the final peptides, of the cysteine residues,

89 2 9 142

6/6 are conserved and of the non-cysteine residues, 0/21 are conserved in the three peptide sequences determined so far.

The results above suggest that the cone snails may have evolved a mechanism for folding the peptide so that the disulfide bonds can be generated independently of the sequences of the final peptide. The data indicate that the excised N terminal region may play an important role in the specific formation of disulfide bonds. Thus, out of the 15 possible different disulfide bonded configurations that can be formed from six cysteine residues, only one (the biologically active form) will actually be generated in vivo.

Thus, in the design of targeted peptide ligands in Conus venoms, it appears that one of the important components for success is to develop an efficient pathway for folding the highly disulfide bonded peptide independently of the final sequence of the peptides. Therefore, such a strategy may also be necessary if targeted peptide ligands are to be generated in vitro.

(The attempt to examine whether targeted peptide ligands can be generated in vitro is only being initiated in the first year grant period. Our plans for the next grant period are summarized below.)

In addition, the 2D-NMR work is continuing and in addition to examining one of the conotoxins specific for the acetylcholine receptor, α-conotoxin SI, we initiated work on the conformation of a novel peptide which induces sleep in young mice, the "sleeper peptide", conotoxin GV. This peptide is unusual among peptides found in Conus venoms in having no disulfide bonds; instead it has five residues of a posttranslationally modified amino acid, γ-carboxyglutamate. The 2D-NMR data on this peptide is presently being collected. As is outlined below, we expect that the structures of these two peptides will be completed in the coming grant period.

Work plan (year 2). We anticipate that the 2D-NMR work for α-conotoxin SI and for the sleeper peptide will be completed in the coming year. We have previously suggested that the sleeper peptide may be folded as an α-helix, and in addition to 2D-NMR data, we plan to also use Raman spectroscopy to examine this question. The role of metal ions, particulary Ca^{++}, in any conformational transitions would also be assessed; this possibility is raised by the presence of the γ-carboxyglutamate residues in the sleeper peptide, which are calcium chelators, important in the mechanism of proteins that have previously been characterized to contain this post-translational modification. Thus, the possibility that calcium or some other ions are involved in the activity of the sleeper peptide is not remote, and any conformational changes induced by divalent ions will be of interest.

In addition, work to see whether new peptides with high affinity for predetermined receptor targets can be generated in vitro will be initiated. The first goal will be to develop an appropriate vector system. In order to do this, the S peptide of ribonuclease will be fused to the lamB protein of Escherichia coli such that the S peptide sequences should be sticking out. The ability of S peptide in such a configuration to stimulate ribonuclease S protein will then be assessed.

Inventions: None

Training Activities: Two postdoctorals were supported by this contract in the first year.

Women or minorities - 1; non-citizens - 2 (citizens of the Philippines).